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And Scientific Research
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Evaluation of the effects of platelet rich fibrin on surgical outcomes after removal of impacted mandibular third molars

A Project submitted to the Scientific Committee of the Department of Oral and Maxillofacial Surgery, College of Dentistry / University of Baghdad, in partial fulfilment of requirements for the BDS degree

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بسم الله الرحمن الرحيم

صدق الله العظيم

Declaration

We- certify that this project entitled "**Evaluation of the effects of platelet rich fibrin on surgical outcomes after removal of impacted mandibular third molars**" by the undergraduate student **Mustafa Maher** under my supervision at the College of Dentistry /University of Baghdad in partial fulfilment of requirements for the degree of Bachelor in Dental Surgery (BDS).

Signature

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Dedication

We dedicate this research to al our professors at Baghdad Col ege of Dentistry in recognition of their great efforts. Also we dedicate it to our families for their unlimited support throughout al these years and to al ourdear friends and col eagues … Final y, our appreciations and thanks to everyone taught us a let er from our childhood until today

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1.1 Blood: An overview

Blood is a bodily fluid that is vital for a number of life functions in animals. To a first approximation, blood is a mildly alkaline aqueous fluid (plasma) in which a large number of free-floating red cells (erythrocytes), white cells (leucocytes), and platelets are suspended**(Atkins, Buckley et al. 2017)**. The primary function of blood is to transport oxygen from the lungs to all the cells of the body and move carbon dioxide in the return direction after it is produced by the cells' metabolism, Blood also carries nutrients to the cells and brings waste products to the liver and kidneys**(Atkins, Buckley et al. 2017)**

The red blood cells are biconcave disc. At the time of their growth, RBCs lose their nuclei and most of their organelles. RBCs can live for about 120 days (Hall 2016) Leukocytes are the cells of the immune system considered the largest blood cells and they are of two types: granulocytes (polymorphoneuclear leukocytes), which include neutrophils, eosinophils and basophils, and agranulocytes, which include monocytes and lymphocytes(Hall 2016) Platelets are non-nucleated minute discs 1-4 micrometers (µm) in diameter, and have half-life in the blood of (8-12) days**(Hall 2016)**

1.1.1 Platelet

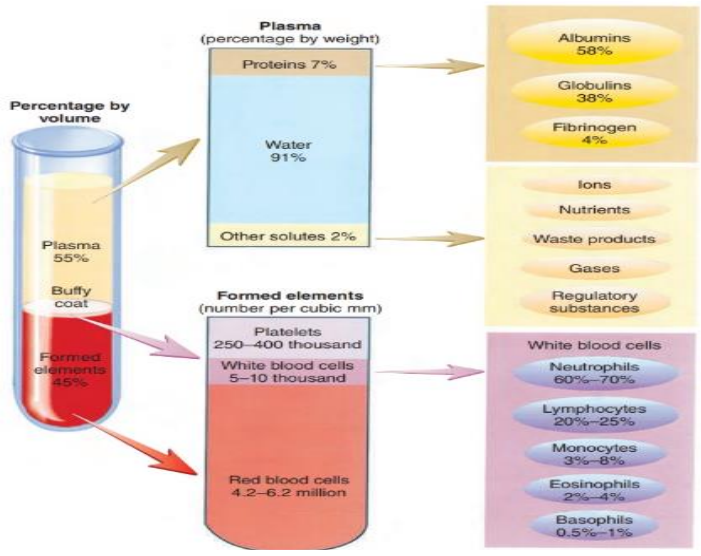


Figure (1.1): Component of blood(Scanlon and Sanders 2018)

Structure

Platelets are the smallest blood cells, The ability of activated platelets to adhere to an injured vessel wall and form aggregates was first described in the 19th century. Besides their long-established roles in thrombosis and hemostasis, platelets are increasingly recognized as pivotal players in numerous other pathophysiological processes including inflammation and atherogenesis, antimicrobial host defense, and tumor growth and metastasis**(Gremmel, Frelinger III et al. 2016)**

Platelets have a complex structure and consist of; microtubules, actin cytoskeleton, mitochondria, Golgi complex, and surrounded by the plasma membrane. From the outermost to the innermost platelets divided into four zones:

The peripheral zone - mostly consists of glycoproteins that are essential for adhesion, activation, and aggregation of platelets, through the numerous receptors that are presents on this glycoprotein layer.

The sol-gel zone - mostly consists of microfilaments and microtubules, which represent the matrix of the platelet that allowing the platelets to maintain their discoid shape.

The organelle zone – mostly contain platelet granules and cellular components such as lysosomes, mitochondria, etc

The membranous zone - consists of membranes organized into a dense tubular system, which is responsible for thromboxane A2 synthesis. The platelets contain invaginations on the plasma membrane referred to as open canalicular system (OCS) from which the platelet products released after platelets activation **(Hall 2016)**

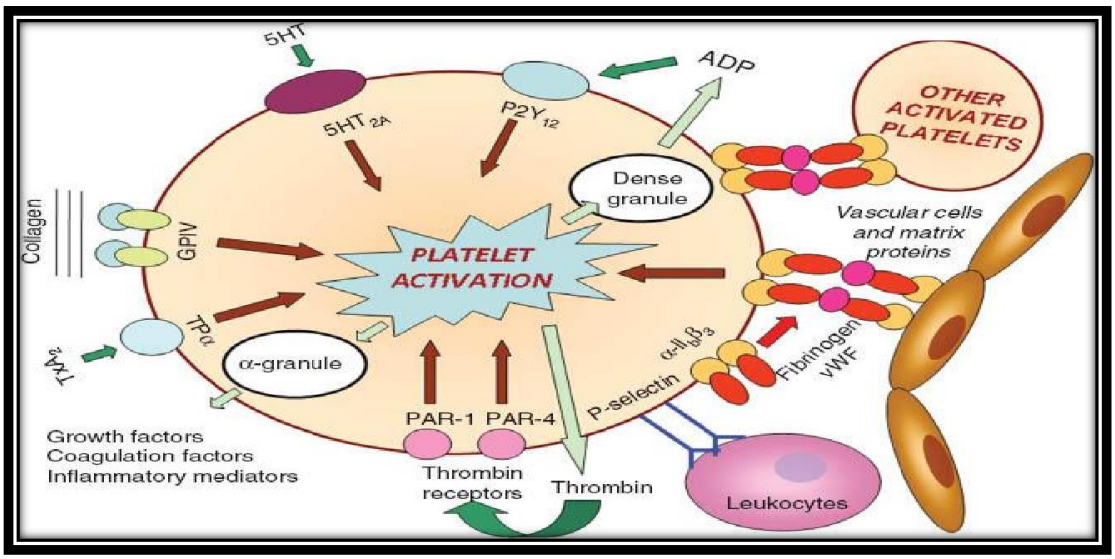
1.1.2 Platelets activations and functions

Platelets play key roles in hemostasis. Platelets are generated from the nucleated precursor cells known as megakaryocytes in the bone marrow and enter the bloodstream without nuclei. Megakaryopoiesis, the complex process that megakaryocytes mature, differentiate, and generate polypoid megakaryocytes, is unique to mammalian cells. Megakaryopoiesis and thrombopoiesis are controlled by multiple cytokines and growth factors, although thrombopoietin is the key regulator. Mature megakaryocytes restructure their cytoplasm and extend pseudopodial projections referred to proplatelets, through cells of the sinusoidal endothelial layer and shed platelets into the circulation.**(Yun, Sim et al. 2016)**

The basic function of platelets is rapidly binding to damaged blood vessels, aggregates to form thrombi, and prevents excessive bleeding. However, activated platelets also aggregate at the site of atherosclerotic plaque rupture or endothelial cell erosion, stimulating thrombus formation and promoting atherothrombotic disease there is an increasing evidence that platelets have a central role in the host inflammation and immune responses **(Yun, Sim et al. 2016)**

Activated platelets stick to each other and to the tissue at the site of injury, creating a platelet plug and catalyzing coagulation cascade reactions that terminate in fibrin creation, clot formation, and organize the consequent inflammatory response and wound healing. These functions are achieved by platelets through expressing and releasing on their surface a diversity of pre-synthesized protein molecules; signaling proteins, membrane proteins, and cytoskeleton regulatory proteins, which are kept internally in the inert platelets**(Ghoshal and Bhattacharyya 2014)**

Normally, the platelets are circulating in their original un activated state through the intact and healthy endothelium of blood vessel, due to the release of prostacyclin (prostaglandin I2) via the healthy endothelium that supports this state, beside the lack of platelet activating factors. The launch of coagulation cascade initiates platelet activity. When blood vessel is damage, the primary target site of platelet action is the subendothelial surface of blood vessel, where the hemostasis establishes. This process entails changes in platelet shape and size. The platelets are activated by thrombin, adenosine Diphosphate (ADP), thromboxane A2 and fibrinogen, these are the soluble agonists that are secreted by the platelets themselves or produced in the coagulation cascade, or by the interfacial agonists such as tissue factor, collagen, or collagen-bound von Willebrand factor (VWF) that become exposed to the blood when vascular endothelium is damaged. Glycoprotein IIb/IIIa (GP IIb/IIIa), von Willebrand factor (VWF), and fibrinogen that are present on the surface of the activated platelet initiate the coagulation cascades. The activated platelets adhere to the collagen under the broken endothelium after binding of VWF by the VWF receptor, also platelets adhere to each other, when fibrinogen bind to (GP IIb/IIIa) forming the “platelet plug” this is known as primary hemostasis, which is reversible. A significant strengthen and positive feedback of the platelet-rich clot are provided by ADP, Thromboxane A2 and other substances such as serotonin, which are released from the activated platelet, initiating the secondary hemostasis that is irreversible. Binding of the prothrombinase complex to the platelet membrane occurs during platelets activation when the platelet membrane phospholipids become negatively charged, converting the prothrombin to thrombin. In this way, a large quantity of thrombin is produced and serves to increase platelet activation and turns fibrinogen to fibrin, forming a fibrin mesh, which traps blood cells. The formed fibrin mesh prolongs the



secondary hemostasis Fig.(1.2).**(Stanger and Kahn 2013, Farré, Modrego et al. 2014, Ghoshal and Bhattacharyya 2014, Yun, Sim et al. 2016)**

Figure (1.2): Platelet activation(Farré, Modrego et al. 2014)

Upon activation, resting platelets are going through exocytosis of intracellular substance from (alpha granule, dense granule, and lysosomes).

α-Granules are unique to platelets and are the most abundant of the platelet granules, numbering 50–80 per platelet. These granules measure 200–500 nm in diameter and account for about 10% of platelet volume. They contain mainly proteins, both membrane-associated receptors (for example, **α**IIbβ3 and P-selectin) and soluble cargo (for example, platelet factor 4 [PF4] and fibrinogen). Proteomic studies have identified more than 300 soluble proteins that are involved in a wide variety of functions, including hemostasis (for example, von Willebrand factor [VWF] and factor V), inflammation (for example, chemokines such as CXCL1 and interleukin-8), and wound healing (for example, vascular endothelial growth factor [VEGF] and fibroblast growth factor [FGF])**(Sharda and Flaumenhaft 2018)**

Platelet dense granules (DGs) are membrane bound compartments that store polyphosphate and small molecules such as ADP, ATP, Ca2+, and serotonin. The release of DG contents plays a central role in platelet aggregation to form a hemostatic plug. Dense granules mainly contain bioactive amines (for example, serotonin and histamine), adenine nucleotides, polyphosphates, and pyrophosphates as well as high concentrations of cations, particularly calcium.**(Ambrosio and Di Pietro 2017, Sharda and Flaumenhaft 2018)**

Platelet lysosomes contain acid hydrolases (cathepsins, hexosaminidase, ß -galactosidase, arylsulfatase, ß -glucuronidase and acid phosphatase) as their most important cargo, and similarly to dense granules they express CD63 and LAMP-1/2. Platelet lysosomal functions have not been well studied. Lysosomes serve a role in the digestion of phagocytic and cytosolic components, similar to that in nucleated cells. Secretion of the lysosomal content may have important extracellular functions, such as supporting receptor cleavage, fibrinolysis and degradation of extracellular matrix components, and remodeling of the vasculature.**(Heijnen and Van Der Sluijs 2015)**

T-Granules it is a novel type of secretory granule has been identified, termed T-granules, given their tubular morphology. T-granules contain TLR9, PDI and VAMP-8 .Platelet spreading on glass and stimulation with type IV collagen increase the surface expression of TLR9, possibly via the SNARE proteins VAMP-8 and VAMP-7. The study suggests that T-granules are recruited to the cell surface and contribute to secretion. However, PDI is a resident ER protein and is exclusively localized to the dense tubular system (DTS)**.(Heijnen and Van Der Sluijs 2015)**

Platelet granules contribute to many aspects of host defense, including hemostasis and thrombosis, inflammation, angiogenesis, and

wound healing. These granules store high concentrations of bioactive cargos in their lumens and contain several receptor types on their membranes.**(Flaumenhaft and Sharda 2019)**

1.2 Platelet concentrates

Platelet concentrates (PCs), mostly represented by platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are autologous biological blood-derived products that may combine plasma/platelet-derived bioactive components, together with fibrin-forming protein able to create a natural three-dimensional scaffold. These types of products are safely used in clinical applications due to the autologous-derived source and the minimally invasive application procedure**(Mariani and Pulsatelli 2020)**

1.2.1 Short History of Platelet Concentrates

The concept of PRP originally was developed in transfusion medicine. In this field, the PRP term was used in 1954 by Kingsley to identify thrombocyte concentrate for treating patients with severe thrombopenia. The history of the techniques to obtain blood-derived products for improving tissue healing started in 1970 with the studies of Matras on fibrin glue use in a rat model.Subsequently, an autologous product termed "platelet–fibrinogen–thrombin mixture" was developed, including, in fibrin glue, a significant concentration of platelets, in order to reinforce the fibrin polymerization In the following years, the role of platelets in supporting tissue healing was confirmed and clinically demonstrated by using a blood-derived product called "platelet-derived wound healing factors or formula-PDWHF" for treating skin ulcers.**(Mariani and Pulsatelli 2020)**

1.2.2 Classification of platelet concentrates

Following the debates about the contents and the role of the various components of these preparations, a first classification was proposed in 2009 and is now widely cited as a milestone in the process of clarification of the terminology. This classification is actually very simple, and separated the products following at least 2 key parameters: the presence of a cell content (mostly leukocytes) and the fibrin architecture. This separation allowed to define 4 main families to regroup the products.

1) **Pure Platelet-Rich Plasma (P-PRP)** – **or Leukocyte-Poor Platelet-Rich Plasma** – products are preparations without leukocytes and with a low-density fibrin network after activation. Per definition, all the products of this family can be used as liquid solutions or in an activated gel form. It can therefore be injected (for example in sports medicine) or placed during gelling on a skin wound or suture (similar to the use of fibrin glues).
2) **Leukocyte-and Platelet-Rich Plasma (L-PRP)** products are preparations with leukocytes and with a low-density fibrin network after activation. Per definition, like the P-PRP, all the products of this family-can be used as liquid solutions or in an activated gel form17. It can therefore be injected (for example in sports medicine) or placed during gelling on a skin wound or suture (similar to the use of fibrin glues).
3) **Pure Platelet-Rich Fibrin (P-PRF)** – or **Leukocyte-Poor Platelet-Rich Fibrin** – are preparations without leukocytes and with a high-density fibrin network. Per definition, these products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix, they can be handled like a real solid material

for other applications. There is only one product in this family, commercially known as Fibrinet PRFM (Platelet-Rich Fibrin Matrix), also marketed for orthopedic applications by Vertical Spine. The main inconvenient of this technique remains its cost and relative complexity in comparison to the other forms of PRF available, the L-PRF (Leukocyte- and Platelet-Rich Fibrin)

4) **Leukocyte- and Platelet-Rich Fibrin (L-PRF) (Choukroun L-PRF)** products are preparations with leukocytes and with a high-density fibrin network Per definition, these products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix, they can be handled like a real solid material for other applications.

This classification system was largely cited, advocated, and validated by a multi-disciplinary consensus conference published in 2012. The POSEIDO (Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization) hold it as its guidelines for all publications on the topic in 2013. This terminology and

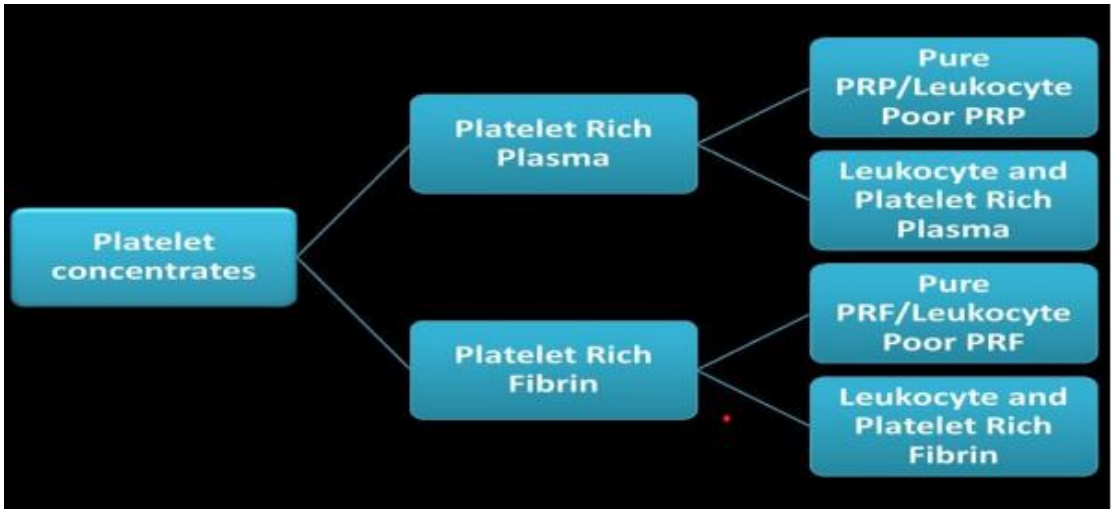


Figure (1.3): Classification of platelet concentrates (Kumar and Gangadharan 2015)

classification are now considered as a basis of consensus in many fields, particularly in oral and maxillofacial disciplines, but many other evolutions may be needed in the future, with more or less relevance depending on the clinical field**(Ehrenfest, Andia et al. 2014)**

1.2.3 (Choukroun L-PRF):

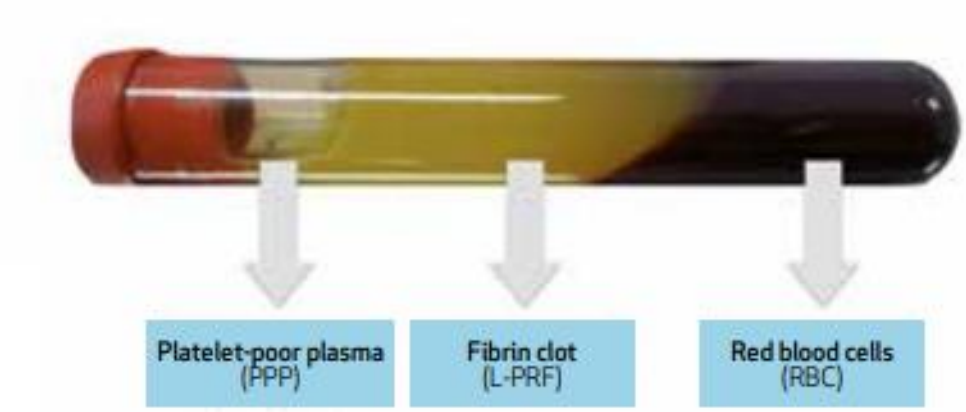
Platelet-rich fibrin (PRF) is a stringently autologous fibrin matrix containing a large quantity of platelets (thrombocytes) and leukocyte cytokines. PRF is an upcoming newer treatment modality used to augment healing process and has been in clinical use over the last decade within many disciplines, such as peri-implant defects, periodontal defects, exclusively as grafting material, in treatment of alopecia, orthopedic, oral-maxillofacial and cardiac surgery, and plastic and cosmetic surgeries. Choukroun in 2001 (France) and his coassociates were among the inventors for use of PRF in any reconstructive surgery to encourage and enhance the bone healing. PRF is found better than the previously used PRP (platelet-rich plasma) as it is easier to prepare and apply, cheaper, and there is no need to add bovine thrombin or anticoagulant in it. Thus in the current setup, it is proving to be benevolent to the surgical reconstructive dentistry.**(Sahu, Jadhav et al. 2020)**

1.2.3.1 Preparation of (Choukroun L-PRF):

The blood collection must be followed by an immediate centrifugation as a prerequisite in the specification of the PRF output. It is formulated to produce a homogeneously-moisturized thick membrane and an exudate rich in platelets, leukocytes, vitronectin, and fibronectin expressed by fibrin clot. Overall, the L-PRF is mechanically resistant, able to support

loads, has a capacity of two times stretching under tension and retains surgical sutures well enough as deforms itself significantly before laceration. The production technique of PRF is very simple and requires only a blood sample and a table centrifuge.**(Crisci, Lombardi et al. 2017)**

The protocol followed is that of "Choukroun *et al*.": the blood samples are collected in 9 mL tubes, without anticoagulant or gel separator, and are immediately centrifuged according to the following program: 30 s



acceleration, 2 min at 2,700 rpm, 4 min at 2,400 rpm, 3 min at 3,000 rpm, and 36 s deceleration and stopping. After centrifugation, three parts are localized in the tube: the red blood cells at the bottom, a fibrin clot that represents the PRF in the middle, and the acellular plasma at the top. We can obtain the PRF extracting the matrix from the tube with forceps and

Figure (1.4) L-PRF tube after centrifugation, with its three compartments(Schär, Diaz-Romero et al. 2015)

removing the red clot. The success of this technique depends entirely on the blood collection and the transfer speed in the centrifuge.**(Crisci, Lombardi et al. 2017)**

The three-centrifugation strata obtained after PRF processing according to the official protocol

A-The acellular plasma or platelet-poor plasma (PPP) that is the topmoststraw colored layer, as the name suggests, is lacking in platelet cells.

B-The L-PRF clot that is rich in fibrin and has the growth factors and cytokines embedded in the polymerized structure.

C-The lower fraction that is red and has the RBC cells**(Schär, Diaz-Romero et al. 2015)**

The speedy blood collection and immediate centrifugation of the blood is advised for successful preparation of PRF, if the blood is not immediately centrifuged, a diffuse polymerization of fibrin occurs, which results in PRF clot of reduced quantity and quality**(Borie, Oliví et al. 2015)**

Ghanaati et al. (2014) formulate a new preparation protocol that is the advanced platelet-rich fibrin (A-PRF), which centrifuged at slower speeds (1500 rpm, for 14 min). In this new form, unlike the original PRF more platelets with T- and B-lymphocytes, stem cells, and monocytes found in the distal part, away from the buffy coat (BC). This new formulation of PRF (A-PRF) when compared to the original PRF, releases significantly higher total quantities of growth factors.**(El Bagdadi, Kubesch et al. 2019)** Recently, Gülnihal Eren et al. (2016) observed that different centrifugation times (10 & 12 minutes) at 2660 rpm in the same centrifugation gravity (400 g) do not have an effect on the platelet and leukocyte counts of the experimental PRF. Therefore, it seems that centrifugation time might not be important for cell counts at a constant gravity, but they observed that after 72 hours, the PRF prepared in 12 minutes retained statistically higher levels of vascular endothelial growth factors (VEGF) than that of the membrane prepared in 10 minutes. The results might suggest that the massive fibrin content of12-minutes PRF-

type membrane is the source for releasing these molecules and could protect growth factors from proteolytic degradation. Additionally Gülnihal reported for the first time, the release of Matrix Metalloproteinase-1 and 8(MMP 1-8) from the PRF that is prepared in 12 minute. These data suggest that PRF prepared in 12 minute via its capacity to increase (MMP-1) and (MMP-8) levels may be able to accelerate extracellular matrix remodeling**.(Eren, Gürkan et al. 2016)**

1.2.3.2 Architecture and Biology of PRF:

Platelet-rich fibrin (PRF) architecture and ultrastructure plays a crucial role in regulating and coordinating the cellular functions and provides a physical architecture, mechanical stability, and biochemical cues necessary for tissue morphogenesis and homeostasis.**(Soares, Babo et al. 2021)**

Both, Fibronectin and Vitronectin are key proteins that play a very important role in the adhesion and migration of the platelets and play a vital role as a key component of the architecture of the fibrin clot. The strong architecture of the fibrin clot formed causes the slow release of the fibronectin over a period of seven days, the fibronectin released initially is the free fibronectin from the exudate and later is replaced by the fibronectin from the PRF membrane. On the other hand, vitronectin is released from the PRF membrane only during the first four hours followed by almost a negligible release over the next seven days Although platelet growth factors play an important role in the biology and functioning of PRF, the fibrin architecture and leukocyte content are two key parameters that have not been looked into in great detail. The fibrin

architecture of the platelet derivatives influences its biology **(Kumar and Gangadharan 2015)**

The pattern of cytokines releasing process in the PRF shows a progressive release for (7-11days) as the fibrin network disintegrates and is different from the fibrin glue enriched with cytokines such as PRP, which will have a massively uncontrollable and short-term effect. Since, PRF results from a natural and progressive polymerization with a three-dimensional organization of the fibrinnetwork, and consists of weak thrombin concentrations resulting in equilateral junctions. These junctions allow the formation of a fine fibrillary network, which can support cytokines enmeshment and cells migration**(Ehrenfest, Andia et al. 2014)**

The PRF clot is The leucocytes that are found in the membrane not only act as anti-inflammatory cells but also as anti-nociceptive, through the release of chemokines and inti-inflammatory cytokines (IL4,IL10,IL-13) and opioid peptides, and thus help in pain control also , an anti-inflammatory cytokine (IL-4), and a key growth promoter of angiogenesis as vascular endothelium growth factor and opioid peptides. Thus, by such content, PRF clots might be considered as an immune organizing node and help in pain control. This could clarify the idea of reduction in postsurgical infection when PRF was used as a surgical additive.**(Ehrenfest, Andia et al. 2014)**

Instead of the different materials used in tissue engineering, PRF seems most promising as the fibrin clot acts as a scaffold, thereby providing mechanical support and serving as biologic connectors. It also, facilitates cellular migration and vascularization. In addition, the presence of the leucocytes in the fibrin network and the slow release of cytokines, results

in self-regulation of the inflammatory, infectious and healing process**(Cortese, Pantaleo et al. 2016)**

1.2.3.3 Platelet and leukocyte distribution in PRF:

in light microscopy light microscopy **(Figure 1.5),** most cell bodies (stained in dark purple for the nuclei) were concentrated in the proximal (head-face) area of each membrane: With Intra-Spin, A-PRF and Salvin, the 3/4 of the cell bodies were observed in the proximal area, and the last 1/4 was observed in the center; the distal part had only residual traces of cell bodies. With LW, the cell bodies appeared more spread all over the membrane (40% proximal, 48% center and 12% distal), as the clot and membranes were particularly small and shrunk. Light microscopy did not allow observing in more details the exact state of these cell bodies. **(Figure 1.5)**. Microscopic evaluation of the PRF membranes produced with the four different centrifuges in light microscopy (hematoxylin eosin). The different membranes showed similar organization in light microscopy, with a concentration of most visible cell bodies (75%) in the first 1/3 proximal part of the membrane (A, x2; B, x80), the remaining in the central 1/3 part (C, x2) and only residual bodies in the last 1/3 distal part (D, x2). Illustration obtained here from an original L-PRF membrane (Intra-Spin). The LW PRF-like membrane was the only one with a

different distribution, mostly due to the strong shrinking of the membrane.**(Dohan Ehrenfest, Pinto et al. 2018)**

1.2.3.4 Types and biological effects of PRF cytokines:

Generally, functional properties of PRP are mainly based on the synthesis and secretion of multiple growth factors that are secreted after platelet activation. These factors are essentially stored in thrombocyte a-granules and they have key role in regulating cellular process, including chemotaxis, mitogenesis and differentiation. Secreted growth factors

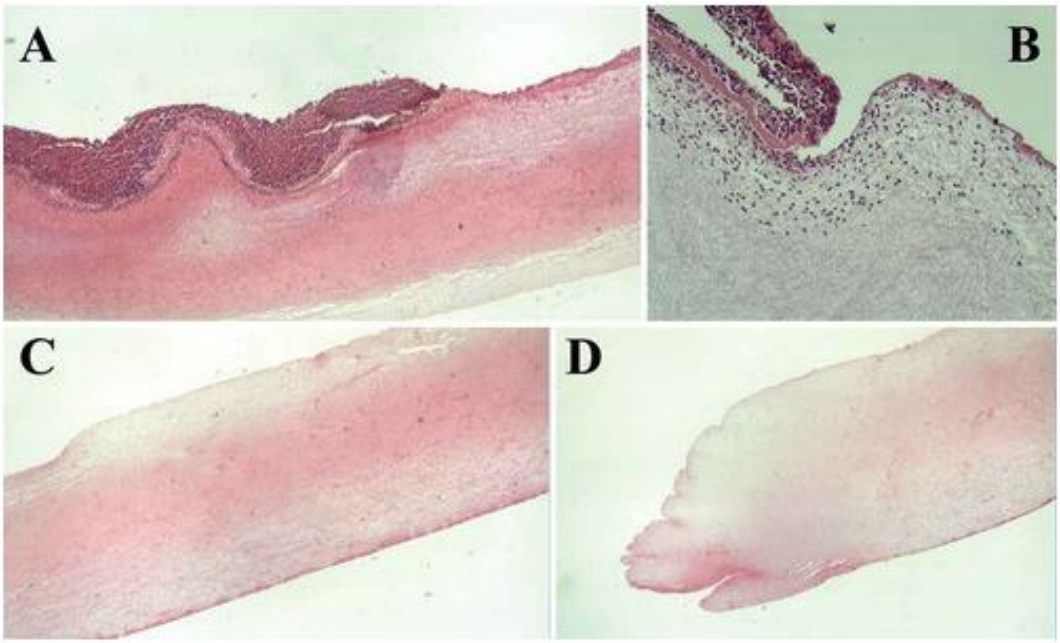


Figure (1.5): Microscopic evaluation of the PRF membranes produced with the four different centrifuges in light microscopy (hematoxylin eosin).(Dohan Ehrenfest, Pinto et al. 2018)

directly stimulate local mesenchymal and epithelial cells to migrate, divide and increase the synthesis of collagen and matrix with resulting formation of fibrous connective tissue and scar formation. Further, many of the growth factors released in damaged tissue express combined action and may also interact between each other, providing the activation of different intracellular signaling pathways with enhanced tissue repair**(Pavlovic, Ciric et al. 2016)**

Growth factor (Gf)	**Function**
Epidermal growth factor (EGF)	• Stimulates cellular proliferation • differentiation of epithelial cells • promotes cytokine secretion by mesenchymal and epithelial cells
Platelet derived growth factor (PDGF)	• Enhances collagen synthesis • proliferation of bone cells • fibroblast chemotaxis and proliferative activity • macrophage activation
Transforming growth factor ß (TGF-ß)	• Enhances synthesis of type I collagen • promotes angiogenesis • stimulates chemotaxis of immune cells • Inhibits osteoclast formation and bone resorption
Vascular endothelial growth factor (VEGF)	• Stimulates angiogenesis • migration and mitosis of endothelial cells • increases permeability of the vessels • stimulates chemotaxis of macrophages and neutrophils
Insulin-like growth factor (IGF)	• Promotes cell growth • Differentiation • recruitment in bone, blood vessel, skin and other tissues • stimulates collagen synthesis together with PDGF
Fibroblast growth factor (FGF)	• Promotes proliferation of mesenchymal cells • chondrocytes and osteoblasts • stimulates the growth and differentiation of chondrocytes and osteoblasts
Tumor necrosis factor	• Growth factor for fibroblasts

alpha (TNFα)	• Promotes angiogenesis • Inhibit tumorigenesis
Interleukin 1 β (IL-1β)	• Inhibits the growth of endothelial cells and hepatocytes • Activates osteoclasts • suppresses the formation of new bone and promotes new bone growth • Enhances inflammatory reactions and collagenase activity
Interleukin 8 (IL-8)	• Supports angiogenesis • Mitogenic for epidermal cells

Table (1.1): The most prominent platelet growth factors, which released from platelets after activation & their functions(Pavlovic, Ciric et al. 2016)

1.2.3.5 Platelet rich fibrin functions & applications:

Platelet-rich fibrin (PRF) is frequently used to accelerate soft and hard tissue healing. The activated platelets in PRF release growth factors, resulting in cellular proliferation, collagen synthesis, and osteoid production. The aim of this study was to compare the stability of dental implants inserted in a one-stage surgical protocol with or without PRF application. Materials and Methods**(Öncü and Alaaddinoglu 2015)**

the effects of platelet-rich fibrin (PRF) exudate on the proliferation, osteogenic differentiation and mineralization of human periodontal ligament cells **(Li, Yang et al. 2018)**

Cell proliferation was enhanced by addition of the PRF exudate, which also promoted the formation of mineralized matrix nodules and upregulated ALP activity and osteoblast-associated levels of osteocalcin**(Li, Yang et al. 2018)**

PRF releases autologous growth factors gradually, express stronger and more durable influence on differentiation and proliferation of osteoblast than PRP, and definitely encourage osseous regeneration over PRP in relations to the density and homogeneity of regenerated bone. In addition, PRF is better than PRP in expression of alkaline phosphatase (ALP) and initiation of mineralization.**(Yelamali and Saikrishna 2015)**

Common growth factors include transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), fibroblast growth factors, insulin-like growth factors (IGFs), and platelet-derived growth factors (PDGFs). These growth factors can improve the osteoinductive properties of bone grafts and stimulate mesenchymal stem cells around the bone grafts to differentiate into chondroblasts or osteoblasts and form new bone.**(Wei, Zhu et al. 2021)**

The main clinical applications of L-PRF and A-PRF include tissue regeneration in oral and maxillofacial surgery (alone or with bone grafts). In regenerative medicine and dentistry, several clinical studies showed better outcomes with PRF than open-flap debridement, in intrabony periodontal defects. Furthermore, its use together with bone substitutes such as nanohydroxyapatite had a therapeutic effect compared with the substitutes alone. Osteonecrosis of the jaws can also benefit from L-PRF application: necrotic bone replacement by L-PRF was used as a physical barrier against microorganisms, preventing secondary infections. PRF can also act in the treatment of ulcers/skin necrosis, plastic surgery, and even musculoskeletal lesions.**(Caruana, Savina et al. 2019)**

In orthopaedic surgery, the use of L-PRF with bone cell proliferation/differentiation inducers such as bone morphogenetic proteins-2 also seems to be a benefit for tissue regeneration. Furthermore, the use of L-PRF in ulcers in a diabetic foot and found that treatment

improved wound healing with no evidence of infection. In other types of chronic ulcers such as pressure and venous leg ulcers, the use of L-PRF has also improved healing and cicatrization.**(Caruana, Savina et al. 2019)**

observed that PRF application appears to increase implant stability as proved by higher implant stability quotient values (ISQ), particularly through the early healing period. Therefore, they stated that application of PRF appears to offer faster osseointegration.**(Öncü and Alaaddinoglu 2015)**

Other clinical applications of PRF:

1) Localized osteitis, 90% of osteitis reduction found in surgical sites of the third molar.
2) Reconstruction of large bone defects after cancer surgery.
3) In plastic surgery, PRF clots used to fill cavities directly or mixed with an adipocyte graft in a lipostructure **(Borie, Oliví et al. 2015)**

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